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A SLURRY BIOCASCADE FOR THE ENHANCED DEGRADATION OF
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ABSTRACT

A slurry biocascade for the degradation of fuels in highly contaminated, weathered, clay-rich soils has been developed. In this biocascade approach, different bacterial populations are optimized for sequential steps in the petroleum hydrocarbon degradation. In the first step of the cascade, the simplest fuel components (e.g., n-alkanes) are biodegraded. Then, the soil is transferred to the next steps in the cascade, in which different "microbial soups" degrade the next groups of hydrocarbons (e.g., the more recalcitrant components, such as multi-ring PAHs). In such a system, each successive step of the cascade maintains a microbial consortium that is optimized to consume organic components of increasing complexity. This "biological chemostat" has been demonstrated for the degradation of total petroleum hydrocarbons (TPH). When compared to the batch approach (in which the microbial population must constantly adapt to a depleting and more recalcitrant carbon source), the biocascade was shown to be much more effective both in terms of the rate and degree of degradation. Pilot-scale studies have demonstrated the feasibility of the cascade biotreatment approach in off-the-shelf commercial bioreactors. The results from these bench and pilot studies and the lessons learned are discussed.

BACKGROUND

The Navy's greatest hazardous waste problem, in terms of total volume of contaminated materials, is soil and sediment impacted by petroleum products. Sites are contaminated primarily by JP-5, a jet fuel, and marine diesel (DFM) from leaking storage tanks and spills from pipelines. Other government and commercial operations also have and continue to generate fuel contaminated sites, both from spills and leaks, generating one of the largest scale hazardous waste problems in the country. Traditionally, treatment of such soils has been limited to dumping or thermal treatment. Such approaches, however, can be very expensive, and are often difficult to implement due to political and regulatory issues. A number of innovative treatment technologies have been developed in recent years, including physical (e.g., separation, thermal treatment, washing), chemical (e.g., reactions or extractions) and biological treatments (e.g., treatments in which organisms are encouraged to convert contaminants to harmless or immobile products).

A petroleum contaminated soil or sediment is a highly complex system. Soils and sediments are combinations of one or many minerals and naturally occurring organic matter, with varying properties such as surface chemistry, grain size and porosity. Petroleum products are mixtures of hundreds of aliphatic and aromatic organic compounds. The relative proportions of each compound vary greatly among fuel types and somewhat between batches of the same type. A careful consideration of these variables is required in order to choose and design intelligent remedial options for such complex mixtures. Due to the unique properties of each fuel and each waste site, there is not one correct remedial approach that can be applied to all contaminants or even all spills of the same contaminant.

For those situations when fines are too abundant for practicable in situ or surface treatment, or when time or space requirements preclude these approaches, slurry bioreactor treatment is a promising alternative. Slurry reactors allow extensive mixing of soils or sediments, providing maximal contact between microbes and contaminants. More rapid degradation can be achieved with reactor systems, due to the higher degree of control maintained over the entire process. Throughput can be maximized by optimizing microbial growth conditions such as air flow, temperature and nutrient levels. Reactor systems handling up to several thousand gallons per unit can be constructed on site or made portable for transport.

Due to its promise for cost-effective treatment of hazardous waste, bioslurry treatment of organic contaminants is the subject of extensive research at government, academic, and commercial

laboratories(1-7). Army Corps, Navy, and EPA SITE program demonstrations have shown bioslurry treatment to be effective for reducing organic contaminants at some sites. Because of the complexity of contaminant-substrate interactions and the site-specific nature of remediation efforts, however, many issues still require resolution. For instance, almost all slurry bioreactor demonstrations published thus far have been carried out in low surface area sandy soils. Little work has been done in more complex, high surface area clay-rich soils, which may concentrate and retain fuel contamination.

APPROACH

Petroleum fuels consist of mixtures of organic molecules, from simple straight-chain alkanes, to multi-ring polycyclic aromatic hydrocarbons (PAHs). Each component differs in its reactivity, solubility(8, 9), volatility, mineral surface affinity(10, 11), and biodegradability(11, 12). Furthermore, different microbial populations or consortia specialize in the degradation of different components or mixtures of components(13). For instance, it has generally been observed that the microbial populations in soils and sediments which have been exposed to contamination for a period of time demonstrate a much better ability to degrade petroleum hydrocarbons(14) and PAHs(15, 16) than microorganisms from pristine soils. It has also been suggested that no single microbial species can degrade mixtures and complex compounds, but rather that these components are degraded by microbial communities or consortia(13). Thus, investigating biodegradation in real, fuel-contaminated soils with native microflora can tell us much more about "real world" biodegradation than can typical experiments with single organic components and individual species of microorganisms.

The objective of this program was to utilize and optimize the capacity of the native microflora to degrade petroleum contaminants. The site used for this study has been contaminated with the jet fuel JP-5 for decades; so, as discussed above, the microbial population has had adequate opportunity to adapt to JP-5 as a carbon source. A sulfur smell was detected at the site when a few mm of surface soil were scraped away, suggesting that natural biodegradation had progressed to the point of anoxia. Thus, *in situ* degradation rates were most likely limited by the availability of an electron donor (*e.g.,* oxygen). In addition, inorganic nutrients (nitrogen and phosphorus) most likely contributed to limited degradation. Slurry bioreactors allow for the controlled addition of these necessary components, allowing the native microflora to metabolize the fuel components.

As discussed above, however, distinct microbial consortia or populations most effectively degrade different components of the complex fuel mixture. Isolation of the microbial communities in our JP-5 degradation studies revealed that the microbial consortia changed as degradation progressed. As the more easily degraded components such as *n*-alkanes were removed from the mixture, different microbes began to dominate the slurry population, presumably those better adapted to utilize the more recalcitrant petroleum components as carbon sources. This shifting of the population progressed through distinct steps, until apparent degradation ceased.

Rather than wait for populations to rise, fall, and shift within a bioreactor, we attempted to develop a series of reactors, in which each reactor contained a rich, active population, optimized for carrying out a separate step in the petroleum degradation process. Once such a series of populations is stabilized, bioreactor throughput can be increased, since the time necessary for progressive generations of microbial populations to shift and grow up is eliminated from the process.

To optimize different populations for successive steps in hydrocarbon degradation, soils are first made into a soil and nutrient-broth slurry and placed in the first slurry bioreactor. The reactor mixes the slurry, supplies a steady stream of air bubbles to provide oxygen, and maintains a minimum temperature. Fuel components are biodegraded to a certain degree (in this case, the *n*-alkanes are allowed to biodegrade). Once this has occurred, half the slurry is transferred to a second bioreactor, and the first reactor is refilled with fresh material. The population in the second reactor is allowed to optimize and degrade more complex compounds such as simple aromatic fuel components. Then, half that material is transferred to the third reactor, and this material is replaced in the second reactor by half the first reactor's material. The first reactor is then again refilled with fresh material. This process is continued until the desired number of bioreactors are set up.

Since only half the material is transferred out of a bioreactor, each reactor retains a slurry charged with a "microbial soup". This "soup" is repeatedly fed organic components of a certain complexity. Through this method, each microbial consortium is optimized to degrade a given group of hydrocarbons. In the early steps, simple components (*e.g.,* *n*-alkanes) are degraded. Later steps in the cascade are optimized for the more recalcitrant components such as multi-ring PAHs. We have called this progressive transfer of soil from one bioreactor to another a biocascade, in which each reactor in the series is a step in that cascade. This "biological chemostat" has been studied at the

laboratory and pilot scale for the degradation of total petroleum hydrocarbons (TPH).

One particularly difficult aspect of using real-world, contaminated materials vs. laboratory-spiked materials for such studies is reduced analytical recovery of fuel components with weathering and time. It is well known that due to sorption processes, analyte recovery decreases with the aging of samples(17-19). This makes mass balance considerations especially difficult. We have also observed that the exposure to surfactants or biosurfactants may enhance contaminant extractability over time. This may confound estimates of degree of biodegradation or loss, since we never really know how much contaminant was there in the first place, or how extraction efficiency changes over time. Spike recoveries do not resolve this problem, since the spike has less time and different conditions under which to interact with the substrate. This must be borne in mind when examining quantitative measures of loss or degradation, however, relative changes are still meaningful. Also, inspection of chromatograms provides qualitative evidence of processes and changes that have occurred.

METHODS

Contaminated soil was sieved through a #10 mesh for the first study and through a #16 mesh for the second and third studies. Slurries were made by mixing one part soil with two parts inorganic nutrient salt solution.

The slurry extraction techniques used in our laboratory have evolved over time as an attempt to minimize the sorption effects described above. Since this paper summarizes the results of experiments carried out over a long period of time, not all samples were extracted in an identical manner.

Samples from all three experiments were extracted in two parts. Slurry samples were centrifuged, and the supernatant and solid were extracted separately. For the first two experiments, 100±30 ml supernatant was acidified and extracted with 10 ml hexane for three minutes. Twenty to forty grams of the solid portion was acidified and extracted with 10 ml hexane for three minutes. Samples from the last experiment were air dried, acidified and extracted with a 1:1:1 mixture of hexane and methanol to soil.

The hexane extracts were analyzed on a Hewlett Packard 5890 gas chromatograph equipped with a cross-linked methyl silicone gum capillary column and a flame ionization detector. TPH concentrations were determined by the internal standard method. All concentrations reported here are the sum of the solid and supernatant phases.

Microbial densities were determined by serial dilutions onto tryptic soy agar plates. Toxicity was measured by using Microtox® on methanol extracts of the slurries. Toxicity is reported as 1/EC20, which is the effective concentration that leads to a 20% decrease in light output of the Microtox® microorganism. A decrease in light output reflects a toxic response to the test material, in this instance, the slurry extract.

To collect and monitor volatile organic carbon (VOC) emissions, the exit ports of the commercial bioreactors were fitted with 60 g charcoal traps. Traps were periodically replaced, and one gram aliquots from the top, middle and bottom of the traps were extracted with 1 ml hexane. The extracts were then analyzed by GC-FID. Total VOC concentrations were extrapolated using an exponential function.

Site description

Work has been carried out on soil from Miramar Naval Air Station, San Diego, CA. The site from which the samples have been collected is a drainage ditch near the taxi way, designated Hangar No. 6. The 240 x 5 m ditch has been contaminated by pulses of fuel, primarily JP-5, from an adjacent fueling station for at least four decades. The ditch is part of a larger drainage system and occasionally receives runoff during storm events.

Soil samples were taken from the ditch in a series of collection trips. In all cases, samples were collected with a shovel to a depth of no more than one foot, and placed in plastic bag lined buckets. Because the purpose of sample collection was to obtain materials suitable for a slurry bioreactor, the preponderance of samples collected were clay and fines-rich, i.e., we avoided sand and gravel.

Even within the confines of a 240 x 5 m ditch, there was great variability in soil mineralogy and degree of contamination. As was stated above, the sampling bias was for soils suitable for the bioreactor, so the samples are by no means representative of all materials in the ditch, but rather of those materials which will most likely be removed and bioslurry treated. Soils were very heterogeneous, including very pure clay lenses, sandy clays, sands and gravels. TPH concentrations of samples, acidified and extracted with 1:1 hexane, ranged from nondetectable to 55,000 ppm. Examination of the GC-FID chromatograms of soil extracts reveals that degree of weathering of the JP-5 in the soils was highly variable (see Figure 3a for three representative soils), and that the volatile fractions

were greatly reduced in some of the more weathered samples (top frame of Figure 3a). The samples with the lowest levels of contamination were often in close proximity to highly contaminated samples. Furthermore, the samples with low or no detectable contamination levels tended to be the very pure, sticky clays, which were so dense and water-saturated as to provide a barrier for petroleum contamination. The highest levels of contamination were in sandy clays, which were primarily high surface area clays, but with enough sand to be permeable.

RESULTS

Flask studies

Initial experiments were carried out in the laboratory, with the "bioreactors" being Erlenmeyer flasks swirled on a shaker table. The flask biocascade initially consisted of six, and later, seven, one liter and 500 ml flasks or steps. The flasks were filled to 40% capacity, and material was transferred from one step of the cascade to another, as described above, until all steps were stabilized. The biocascade was split and maintained for over a year to verify sustainability. Figure 1a shows the results of this experiment. For the first 120 days of the experiment, the cascade was split every week, through six steps. During this time, starting material placed in the first step of the cascade had an extractable TPH concentration of ~9.95 mg/g (9950 ppm). After day 120, split frequency was reduced to once every two weeks, to determine if the degree of degradation would improve with longer residence times. During this time, starting material placed in the first step of the cascade had an average extractable TPH concentration of 5.8 mg/g (5800 ppm).

When the cascades were split, samples for analysis were taken from Flasks 2 and 6, and later, from Flask 7. In the first 120 days, samples from Flask 2 had a one week residence time in Flask 1 and one week in Flask 2, for a total of two weeks in the slurry cascade. Flask 6 had a total of six weeks in the cascade (one week in each flask), or 28 days since the soil had been in Flask 2. After day 120, samples taken from Flask 2 had two weeks each in Flask 1 and 2, or a total of 4 weeks residence time, while Flask 7 had 14 weeks residence time, or 70 days since that material had been in Flask 2.

As is true for remediation efforts in the field, the material put through the cascade was variable and heterogeneous, and this accounts for a large part of the variability observed in Figure 1a. However, several important features can be pointed out. First, the biocascade remained viable and sustainable for well over a year. Second, longer residence times resulted

in more extensive degradation (as observed in the TPH change in Figure 1a at day 120, when splits changed from once per week to once every two weeks). Third, degradation did not appear to bring contamination levels to zero - rather, they seemed to level off at about 100 ppm.
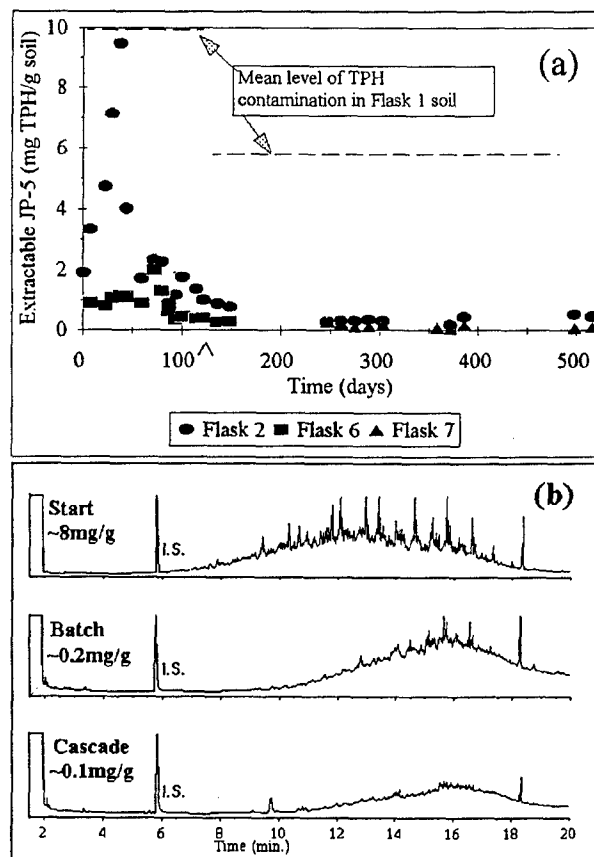

Figure 1. (a) Results of the flask biocascade experiment. ^ indicates switch from weekly to biweekly splits. (b) GC-FID chromatograms of extracts of starting soil, and soil after 14 weeks in the batch and biocascade reactors. See text for discussion.

It was also important to compare degradation rate and efficacy in the biocascade to that in a simple batch reactor. So, in parallel with the injection of material into the stabilized biocascade, a slurry was left in the batch mode. Figure 1b contains GC-FID chromatograms of extracts of the starting material and the material after 14 weeks in the biocascade and the batch reactor. The chromatograms of the cascade and batch products differ in important ways. First, the biocascade material has fewer and smaller peaks standing above the unresolved complex mixture (UCM), or hump of the chromatogram. This suggests that the biocascade more effectively biodegraded more recalcitrant fuel components. Furthermore, the degree of degradation was greater in the biocascade: 100 ppm TPH remaining from an original level of 8000

ppm, compared to 200 ppm remaining in the batch reactor.

Thus, the cascade approach was shown to be much more effective than batch biodegradation both in terms of rate and degree of degradation. As a result of evaluation of several flask studies, it was determined that for the material being used here, three biocascade steps were sufficient, so the subsequent pilot studies were carried out in a three-step biocascade.

Pilot studies

The feasibility and efficacy of the biocascade in pilot-scale units was tested next. The commercial bioslurry units had 75-l. capacities, with an impeller, rake and slurry lift system to keep the materials in a slurry suspension, and air diffusers to infuse oxygen into the system. The units were thermally insulated and the minimum temperature could be controlled. The temperature was set to 72°F, but since the work was being done in San Diego, the temperatures often reached the upper 80s by late afternoon.

Figure 2, a and b, shows the results of one pilot study. In this study, samples were taken from the reactor spigots on a regular basis, so concentrations were monitored over time and at times other than during a split. Reactor volume allowed for sampling without significantly impacting total volume. The symbols show extractable TPH concentrations over time for samples from all three steps in the biocascade. Figure 2a shows that three splits occurred at days 8, 16 and 31 (as indicated by the vertical lines). At each split, Step 1 received an input of fresh material, as depicted by the high spikes in Step 1 data. As has been discussed, the material from this site was highly heterogeneous in mineralogy, contaminant concentration and degree of weathering. This can be seen in the variable TPH concentration of material in Step 1 after each split. Splits were not carried out at uniform time periods for several reasons: degradation to designated levels took a variable period of time with variable materials, and operational difficulties were encountered with the bioreactor units (briefly discussed in a subsequent section).

Several important features can be seen in Figure 2a. The most rapid degradation (of the simplest fuel components) happens in Step 1. Degradation continues in Step 2, but of more complex components, so at a much slower rate than in Step 1. In Step 3, the lowest levels, about 100 ppm, are reached. Although it is difficult to see in the low values of Step 3 in Figure 2a, TPH concentrations steadily drop with time in the Step 3 bioreactor. Once the approximate level of 100 ppm is reached, though, no clear evidence of further degradation is observed.
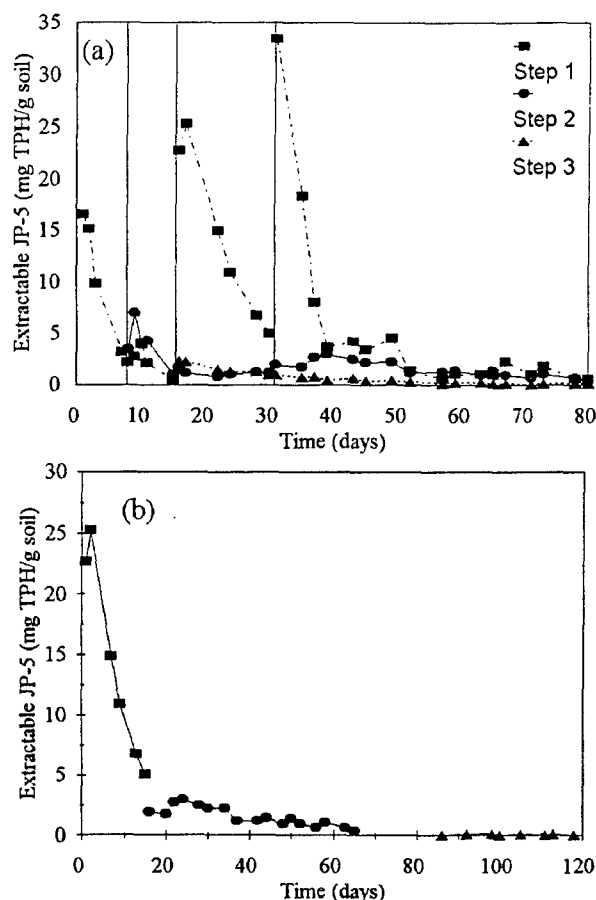

Figure 2. (a) Results of the pilot slurry biocascade. Vertical lines indicate splits. (b) Detail of the third slug of material as it travels through three steps of the slurry biocascade.

Figure 2b follows the third slug of material through the three steps of the biocascade. It should be pointed out, however, that it is impossible to truly track the TPH concentration of a discrete slug of material from one step to another, since only 50% of the material is transferred, and once it is transferred, it is immediately diluted by the slurry remaining in the receiving reactor. Also, for very heterogeneous materials, it is possible that the TPH concentration of the material being transferred is lower in concentration (but not degree of degradation) than that in the subsequent reactor. Concentrations would then appear to rise between successive steps. Given these caveats, one can see why the data are not perfectly smooth in Figure 2. However, one can still see continuous degradation of JP-5 components as material moves through the biocascade.

To address the effects of material heterogeneity in the pilot bioreactors, three bioreactors, containing different soils, were run in parallel in the batch mode. Figure 3a shows the GC-FID chromatograms of extracts of materials placed in the three reactors. Reactor 1's soil shows extensive weathering - most of

the spikes in the chromatogram, which generally represent straight-chain and branched alkanes, are very low. Also, the peak of the UCM is shifted to the right, suggesting that the more volatile components (generally reflected in the early eluted, left-hand side of the chromatogram) are missing. Reactor 3 materials, on the other hand, appear very fresh, with the GC-FID trace looking very much like fresh JP-5. This is not to say, however, that older, weathered material may not be in this sample, but rather that its signature may be buried in the signal of much fresher fuel. Reactor 2's soil lies somewhere between these extremes. While the percentage fines varies somewhat between these three batches of soil, they are all at least 50% fine-grained material, and, thus can all be considered fines or clay-dominated systems.

Figure 3b shows extractable TPH values for samples taken from the three bioreactors over time. The fresher materials in Reactor 3 start at much higher TPH levels, about 14,000 ppm, than do Reactors 1 and 2, which start at about 6000 ppm. Not surprisingly, the fresh Reactor 3 material is rapidly degraded. Reactors 1 and 2 also show evidence of rapid degradation, but not nearly as rapid as that in Reactor 3. By about day 10, however, TPH levels in the three reactors converge, and degradation rates proceed at a slower rate.

Figure 3c shows the microbial activity and toxicity of the materials in the reactors during the experiment. Microbial densities, shown in log colony forming units (CFU) per ml, rise rapidly in the first few days, due to the added inorganic nutrients and adequate oxygen, and then remain at a relatively healthy level throughout the experiment.

Toxicity, as measured by Microtox® measurements of a methanol extract, dropped rapidly in the first few days in the slurry, suggesting that biodegradation may rapidly reduce acute toxicity of the fuel-contaminated soils. Since we have not examined which fuel components were causing the Microtox® response, we cannot comment on what was causing the toxicity reduction. It should be noted, however, that the Microtox® organisms were sensitive to methanol, and thus all extracts had to be diluted by a factor of 10 to run the toxicity assays. For this reason, toxicity values which have dropped to zero on the graph have actually just dropped below the detection level of the assay.

Throughout this experiment, air flow out of the reactors was diverted through charcoal traps. These traps were periodically removed, and the charcoal was sectioned and extracted. The extract was analyzed for TPH concentration and composition. Hydrocarbon components were always detected in the extract from
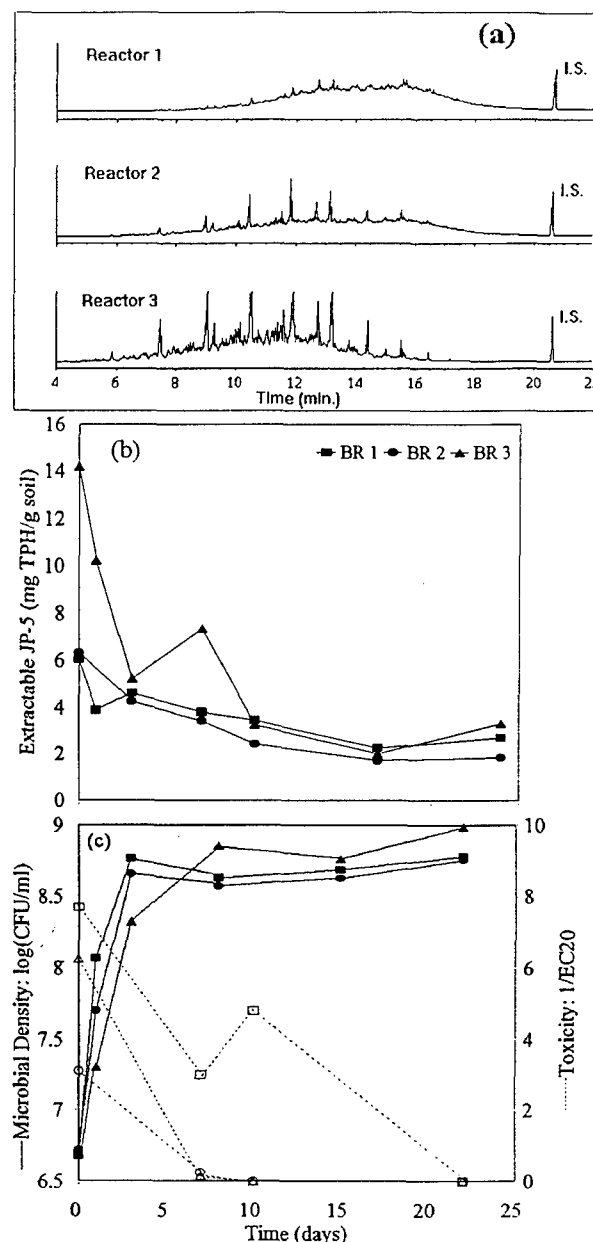

Figure 3. (a) GC-FID chromatograms of extracts of soils placed in the three bioreactors. (b) Extractable JP-5 concentrations and (c) microbial densities and toxicity of slurries removed from the bioreactors over time.

the top section of the trap, and thus it cannot be guaranteed that some breakthrough had not occurred. However, the traps were never saturated, and TPH concentrations in the lower portion of the traps were always much higher than in the upper portion, suggesting that the bulk of the hydrocarbons exiting the reactors were retained by the traps. Values reported for volatile loss, which were extrapolated from an exponential function, were not very different from total trapped values. Nevertheless the volatile loss values must be considered minimum numbers, but are most likely close to actual values. It should be

noted that air flow through the reactors proved to be highly variable and difficult to measure or control. Therefore, air flows were not necessarily the same in all three bioreactors.

Based upon the TPH values measured in slurry extracts in the three reactors over time, the total TPH losses relative to the original concentrations in the three reactors can be calculated to be 55, 74 and 82%, respectively, over the 24 days of the experiment. The greatest loss was from Reactor 3, the reactor with the freshest material.

Based upon the charcoal trap extractions, the minimum losses to volatilization and bubble stripping were 2, 1 and 4%, respectively, for Bioreactors 1, 2, and 3. These values are in contrast with values of 60-90% which we have observed for volatile loss during biodegradation of fuels in water. A possible explanation for the low amount of volatile loss in spite of constant bubble stripping is the fact that the slurries were so clay rich. With a typical clay having a surface area of 20-100 m^2/g, the material in the bioreactors had 0.5-6 *million* square meters of surface area on which the fuel components could partition. This would be expected to inhibit air stripping to some degree.

At the beginning of the study, the fuel components caught in the trap represented the lighter components of the soil contaminants. As the remediation process continued, heavier hydrocarbons were apparent in the trap extracts, because advanced degradation and volatilization had previously depleted the simpler, light-end compounds.

By subtracting the loss due to volatilization from the total loss, a maximum percent loss due to biodegradation (or other undetermined abiogenic processes) can be calculated. These values are 53, 73 and 78%, respectively, for Reactors 1, 2, and 3. These are very high loss values that can be attributable to actual destruction of the contaminants, rather than loss to the atmosphere.

OPERATIONAL CONSIDERATIONS

Bioreactors

The 75-l. bioreactor units used in the pilot studies were EIMCO Biolift Reactors. During the pilot and feasibility studies, a number of operational problems were encountered which were specific to reactor design and not to bioslurry treatment itself. At times, operations were halted as the problems were addressed. In the interest of helping future workers avoid and/or design for these problems, they are briefly described below.

Probably due to the thick, clayey nature of our soils, air lifts and air diffusers often clogged. Possibly also due to the thick "milk-shake" consistency of the slurry and a buildup of microbially generated organic matter that had a tendency to foam, a slurry/foam mixture would exude up from the reactor around the mid shaft in the motor housing. This foaming apparently caused "explosions" in which slurry would shoot out of any and all openings of the reactor. Some of the airlift and diffuser clogging problems could have been reduced by an increase in airflow, but this also exaggerated the "explosion" problems. Vents and traps to catch overflow were placed at the top of the reactors, but the interrelated clogging and venting problems were never satisfactorily resolved. Based upon these and other problems, the vendor has redesigned the pilot units and equipped them with foam breakers. We have not, however, verified whether these changes resolved the problems. At the other mineralogical extreme from clay, coarse-grained sands tended to settle to the bottom, resulting in a stratified rather than homogeneous slurry. A method for generating a slurry mixture of the proper consistency to prevent stratification was suggested by the vendor, but it was not practicable for the soil being treated.

As has been mentioned before, air flow was variable and hard to control. It was also discovered that the flow gauges in no way reflected actual flow. This problem was never resolved.

Although the three bioreactor units were set up and designed for "continuous flow" use, in which material could flow from one reactor down to the next, attempts to use this set-up for transferring material from one reactor to another during a cascade split met with difficulties. Material pumped into a reactor simply flowed over the slurry in that reactor and ended up in the next reactor. To achieve a proper split, we pumped material out of the reactors into buckets, and then pumped the slurries into the next reactors. This would not have been an acceptable solution for a larger-scale project, and must be resolved before scale-up. The reactors were also extremely difficult to completely empty and clean, since there was no outlet at the bottom. Newer units now have a bottom spigot. Sampling valves had a right-angle configuration which often clogged. Valves were replaced in-house with a more practical design.

Impeller motor brushes also burned out frequently (every 300-400 hours of operation) and needed to be replaced. Carbon built up in the motors as a result, ultimately causing circuit breakers to switch. While brush replacement and motor cleaning could be considered regular maintenance, it could become a significant factor in a long-term treatment.

Discussions with other workers using slurry bioreactors revealed that such problems are often encountered. However, all these engineering problems are surmountable, and, it has been suggested (*e.g.,* personal communication, Mark Zappi, Waterways Experiment Station) that the 75 l. size bioreactors are the most difficult to deal with, and that larger units are less problematic.

VOC emissions during sample handling

In order to determine potential exposure of workers to volatile organic carbon (VOC) emissions during sample handling, VOCs were measured with photoionization detectors (PIDs) during all aspects of sample treatment: from collecting soil to preparing it for the bioreactor. During sampling trips, the average readings for all areas of the site at waist level were low. The highest VOC concentration detected at waist level was 2.3 ppm.

Before being placed in the bioreactor, all soils were wet sieved. Soils had been stored in buckets prior to sieving. The dry weight normalized TPH concentration of the bulk soil was 700 - 800 ppm. After sieving, the TPH concentration of the wet slurry soil was 2100 ppm. The slurry concentration is higher than the bulk soil, because the fine-grained material, which tends to hold more contaminants, was concentrated during sieving.

Although the VOC levels measured at workers' face level were not very high, a fuel smell could be observed at all times. There was a light breeze, and the temperature was about 21.5°C. All work was done in the shade. Face level readings were between 0 and 10 ppm at all times, except when the bucket was first opened (25-50 ppm) and when measurements were taken directly in the bucket, and when the slurry was stirred. Slurry transfer probably leads to relatively high VOC levels, but measurements were not taken during transfers.

VOC emission during soil treatment should vary as a function of soil TPH content, mineralogy, temperature, breeze, and other factors. These results should only represent an estimate of emission for a particular operation.

CONCLUSIONS

In these experiments, the biocascade approach was shown to be much more effective than batch biodegradation both in terms of the rate and degree of degradation. Pilot-scale studies have demonstrated the feasibility of the cascade biotreatment approach in off-the-shelf commercial bioreactors. Biodegradation of JP-5 contamination in highly weathered, clay-rich soils

was shown to proceed to levels of about 100 ppm in a biocascade, as opposed to ~200 ppm in the batch mode. This cascade approach reduces the time ordinarily required for microbial populations to shift in response to a depleting and progressively more recalcitrant carbon source, and thus can increase throughput.

ACKNOWLEDGMENTS

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